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IS 10269 (1982): Haemocytometer Counting Chambers Clinical and Diagnostic Apparatus [MHD 12: Hospital Equipment]

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“Knowledge is such a treasure which cannot be stolen”





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*Indian Standard*  
SPECIFICATION FOR  
HAEMOCYTOMETER COUNTING  
CHAMBERS CLINICAL AND  
DIAGNOSTIC APPARATUS

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INDIAN STANDARDS INSTITUTION  
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG  
NEW DELHI 110002

# *Indian Standard*

## SPECIFICATION FOR HAEMOCYTOMETER COUNTING CHAMBERS CLINICAL AND DIAGNOSTIC APPARATUS

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***Indian Standard***

SPECIFICATION FOR  
HAEMOCYTOMETER COUNTING  
CHAMBERS CLINICAL AND  
DIAGNOSTIC APPARATUS

O . F O R E W O R D

0.1 This Indian Standard was adopted by the Indian Standards Institution on 28 June 1982, after the draft finalized by the Medical Glass Instruments and Appliances Sectional Committee had been approved by the Consumer Products and Medical Instruments Division Council.

0.2 For a long time a need was felt to formulate a national standard for haemocytometer counting chambers. In this standard besides one ruling and two ruling, four ruling chamber has also been included. The four ruling chamber is suitable for leucocyte counts, but not for red-cell counts because of drying out of the sample in the fourth and possibly also third ruled area during counting of the first and second areas. The tolerances on dimensions specified in this Indian Standard provide for the highest degree of accuracy which is considered to be attainable in manufacture without unduly increasing the cost of production. Nevertheless, it is important to note that errors as large as  $\pm 7$  percent on a blood count could occur if, by chance, all the tolerances permitted by this standard for different parts of the apparatus were cumulative. Therefore, for work where greater accuracy than this is required, it is advisable to determine the accuracy of the apparatus by direct measurement of its dimensions, or other suitable method.

0.3 In the preparation of this standard considerable assistance has been derived from BS 748: 1963 haemocytometer counting chamber and dilution pipettes published by the British Standards Institution.

0.4 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test, shall be rounded off in accordance with IS : 2-1960\*. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

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\*Rules for rounding off numerical values ( revised ).

## 1. SCOPE

**1.1** The standard specifies three types of haemocytometer counting chamber with one, two or four rulings and four types of ruling.

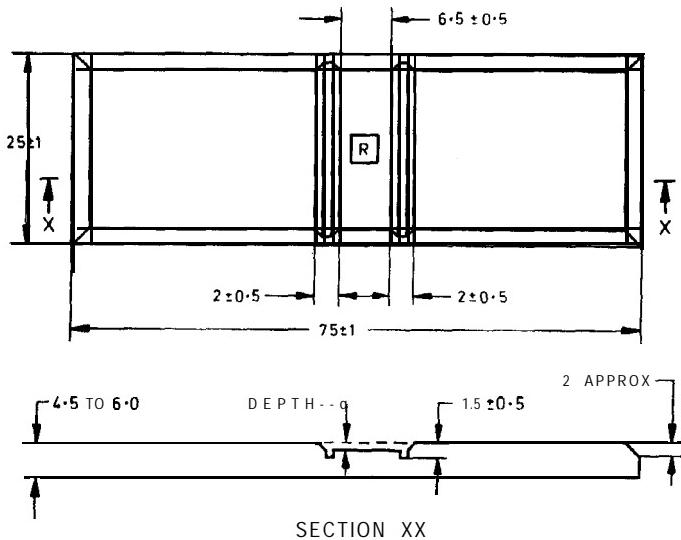
## 2. TYPES OF CHAMBER

**2.1** The counting chamber shall consist of a recess in a glass slide bridged by a cover-glass. The base of the recess shall bear either one, two or four rulings of one of the types specified in 8.

**2.2** If required by the purchaser the counting chamber may be provided with metalized lines (bright lines), satisfying the requirements specified in 8.4.

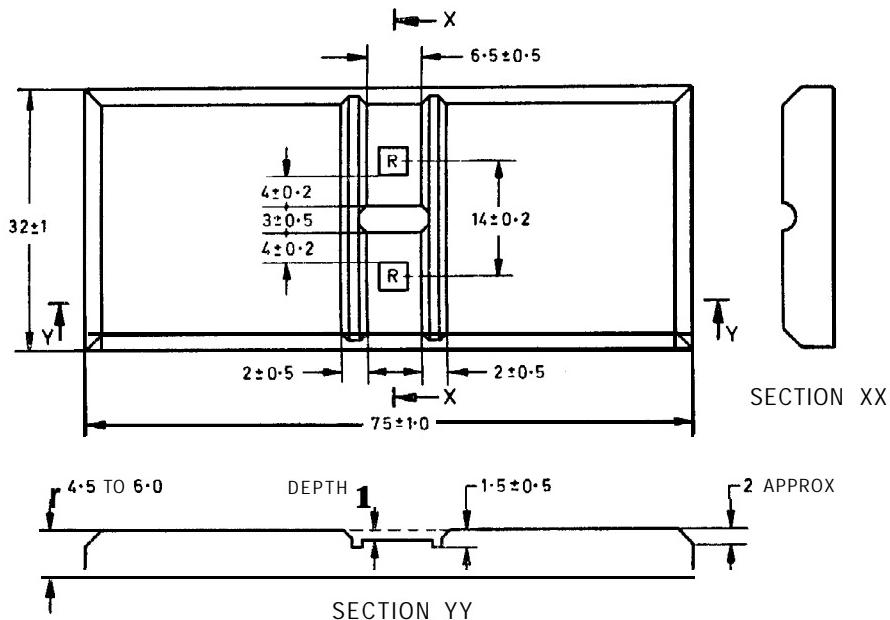
## 3. DESIGN AND DIMENSIONS

**3.1** The general design of the glass slides is shown diagrammatically in Fig. 1, 2 and 3.



All dimensions in millimetres.

**FIG. 1 HAEMOCYTOMETER COUNTING CHAMBER WITH ONE RULING**



All dimensions in millimetres.

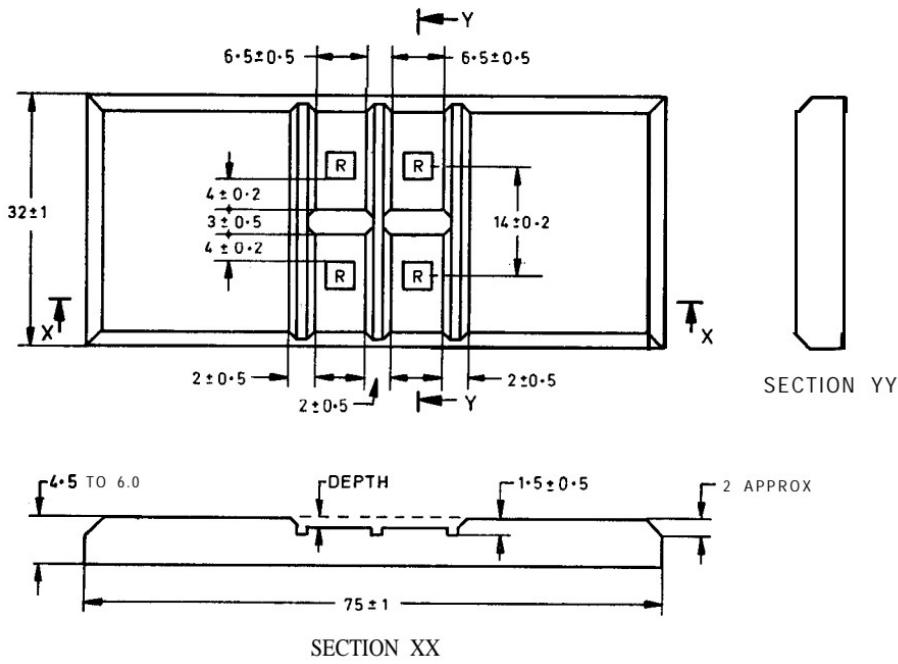
**FIG. 2 HAEMOCYTOMETER COUNTING CHAMBER WITH TWO RULINGS**

3.2 The glass slides shall conform to the dimensions given in Table 1. The upper edges of the slides shall be **bevelled** as shown in Figures 1 to 3. The lower surface of the slide may, if required, have concavity below the ruled area or areas so as to minimize the danger of the lower surface becoming scratched.

**TABLE 1 DIMENSIONS OF SLIDES**

(*Clause 3.2*)

TYPE OF CHAMBER	LENGTH mm	BREADTH mm	THICKNESS mm
One ruling	$75.0 \pm 1.0$	$25.0 \pm 1.0$	4.5 to 6.0
Two ruling	$75.0 \pm 1.0$	$32.0 \pm 1.0$	4.5 to 6.0
Four ruling	$75.0 \pm 1.0$	$32.0 \pm 1.0$	4.5 to 6.0



All dimensions in millimetres.

**FIG. 3 HAEMOCYTOMETER COUNTING CHAMBER WITH FOUR RULINGS**

#### 4. TRANSVERSE GROOVES

4.1 Slides with one or two rulings shall have two transverse grooves parallel to the shorter edges as shown in Fig. 1 and 2. Slides with four rulings shall have three such grooves, as shown in Fig. 3. The edges where the sides of each groove would meet the surface of the slide shall be slightly bevelled.

4.2 The width of each groove measured on the surface of the slide to the outside edges of the bevels, shall be  $2.0 \pm 0.5$  mm.

4.3 The depth of each groove shall be  $1.5 \pm 0.5$  mm measured from the surface of the slide.

4.4 The grooves shall be symmetrically disposed about the middle of the slide.

4.5 The plane surface of the slide between the bevels of adjacent grooves shall measure  $6.5 \pm 0.5$  mm.

## 5. LONGITUDINAL GROOVE

**5.1** Slides with two or four rulings shall have a longitudinal groove of semi-circular cross-section connecting the centres of the transverse grooves as shown in Fig. 2 and 3.

5.2 The width of this groove measured on the surface of the slide shall be  $3.0 \pm 0.5$  mm.

## 6. FLATNESS OF UPPER SURFACE OF SLIDE

**6.1** When tested conjointly by means of an optical flat, the area of the slide which in use is normally covered by the cover glass shall show not more than three interference fringes in the green light from a mercury vapour lamp.

6.2 The ruled areas between the grooves shall be flat and parallel to the plane of the two outer areas, to within 0.001 mm.

## 7. DEPTH OF CHAMBER

7.1 For neubauer, improved neubauer and biirker rulings, the mean depth of the areas between the grooves below the plane of the two outer areas shall be  $0.100 \pm 0.001$  mm.

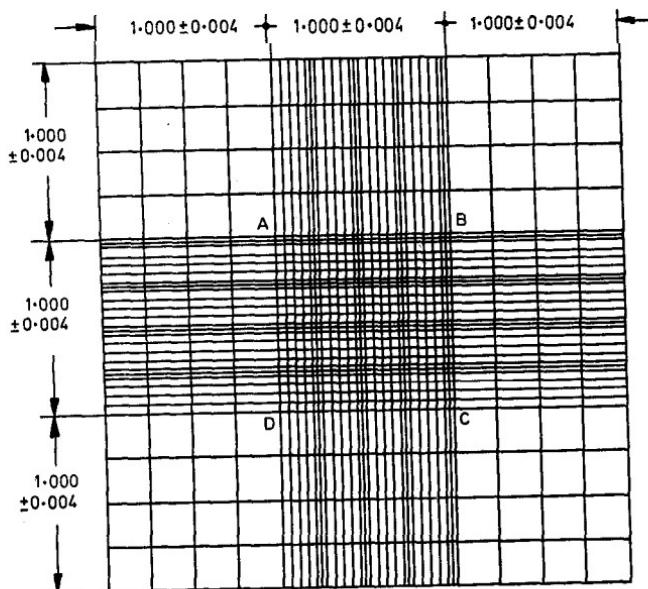
7.2 For modified Fuchs-Rosenthal ruling, the depth shall be  $0.200 \pm 0.001$  mm. except for the four-ruled chamber in which the depth shall be  $0.100 \pm 0.001$  mm.

## 8. RULINGS

**8.1 Position** — The ruling shall occupy the position marked 'R' in Figures 1 to 3 and shall be centrally placed between the transverse grooves subject to a tolerance of  $\pm 0.4$  mm.

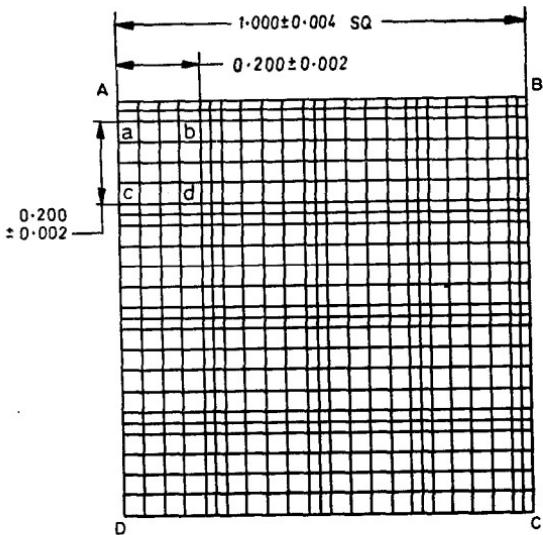
**8.2 Type** — The ruling shall be one of the following types :

- a) **Neubauer** — This ruling is shown in Fig. 4. Each of the nine large squares into which the ruling is divided has sides 1 mm in length. The central square **ABCD** of Fig. 4, shown on a larger scale in Fig. 5, is subdivided into 16 squares, such as **abcd**, each of which in turn contains 16 squares having sides 0.05 mm in length. Variations are permissible in the ruling of the four corner squares around the central one, but if a ruling of these squares different from that shown in Fig. 4 is required, details shall be specified by the purchaser. Mirror images of the ruling shown in Fig. 4 are permitted.



All dimensions in millimetres.

**FIG. 4 NEUBAUER RULING**



All dimensions in millimetres.

**FIG. 5 CENTRAL PORTION OF NEUBAUER RULING**

- b) **Improved Neubauer** — This ruling is shown in Fig. 6. Each of the 9 large squares into which the ruling is divided has sides 1 mm in length. The central square **ABCD** of Fig. 6, shown on a larger scale in Fig. 7, is subdivided into 25 squares such as **abcd**, each of which in turn contains 16 squares having sides 0·05 mm in length.
- c) **Biirker** — This ruling is shown in Fig. 8. Each of the 9 large squares such as **ABCD** into which the ruling is divided has sides 1 mm in length. Each of these large squares contains 16 small squares such as **abcd**, having sides 0·2 mm in length, the subdivision being effected by pairs of lines 0·05 mm apart.
- d) **Modified Fuchs-Rosenthal** — This ruling is shown in Fig. 9. Each of the nine large squares such as **ABCD** into which the ruling is divided has sides 1 mm in length. These squares are indicated by a triple ruling approximately 0·05 mm wide. Each of these large squares is divided into 16 small squares, such as, **abcd**, having sides 0·25 mm in length, the subdivision being effected by single lines.

**NOTE** — The counting areas used should be areas such as **abcd** for red corpuscles or **ABCD** for white corpuscles, and not the remaining small squares or rectangles. This applies to all rulings except the Modified Fuchs-Rosenthal which is used for counting white corpuscles only.

### 8.3 Accuracy

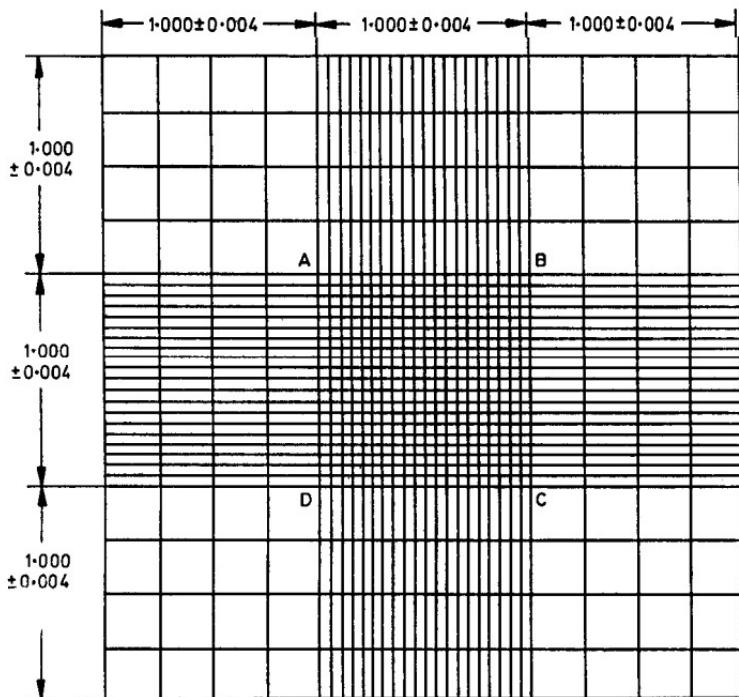
- a) In each type of ruling the length of each side, of each square such as **ABCD** shall be  $1.000 \pm 0.004$  mm.
- b) The length of each side of each square such as **abcd** shall be  $0.200 \pm 0.002$  mm in Neubauer, Improved Neubauer and Bürker rulings; and shall be  $0.250 \pm 0.002$  mm in Modified Fuchs-Rosenthal ruling.
- c) The lines shall be sufficiently fine and uniform in thickness to enable measurements to be taken to an accuracy compatible with the above tolerances.

### 8.4 Metallized Markings and Films

— Any metallized markings or films shall be unaffected by contact with 2 percent acetic acid under normal conditions of use.

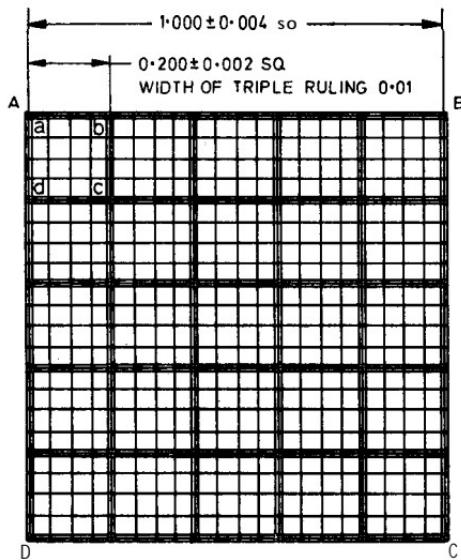
9. Normally two cover-glasses are supplied by the manufacturer of the counting chambers. When they are supplied along with the counting chambers, they shall comply with the requirements specified in 9.1 to 9.6.

9.1 The cover-glasses shall be well-annealed and free from any defect which would interfere with clear vision through the glass when viewed through a microscope.



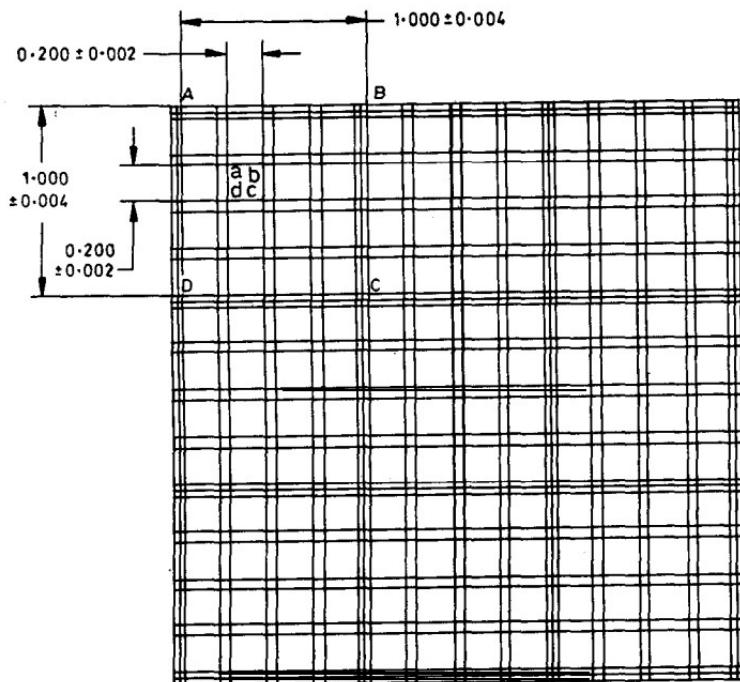
All dimensions in millimetres.

FIG. 6 IMPROVED NEUBAUER RULING



All dimensions in millimetres.

FIG. 7 CENTRAL PORTION OF IMPROVED NEUBAUER RULING



All dimensions in **millimetres**.

**FIG. 8 BÜRKER RULING**

**9.2** The corners shall be rounded.

**9.3** Cover-glasses for slides with one ruling shall be 22 x 16 mm, for those with two rulings 25 x 22 mm and for those with four rulings 35 x 25 mm. The tolerance on each of these dimensions shall be  $\pm 1$  mm.

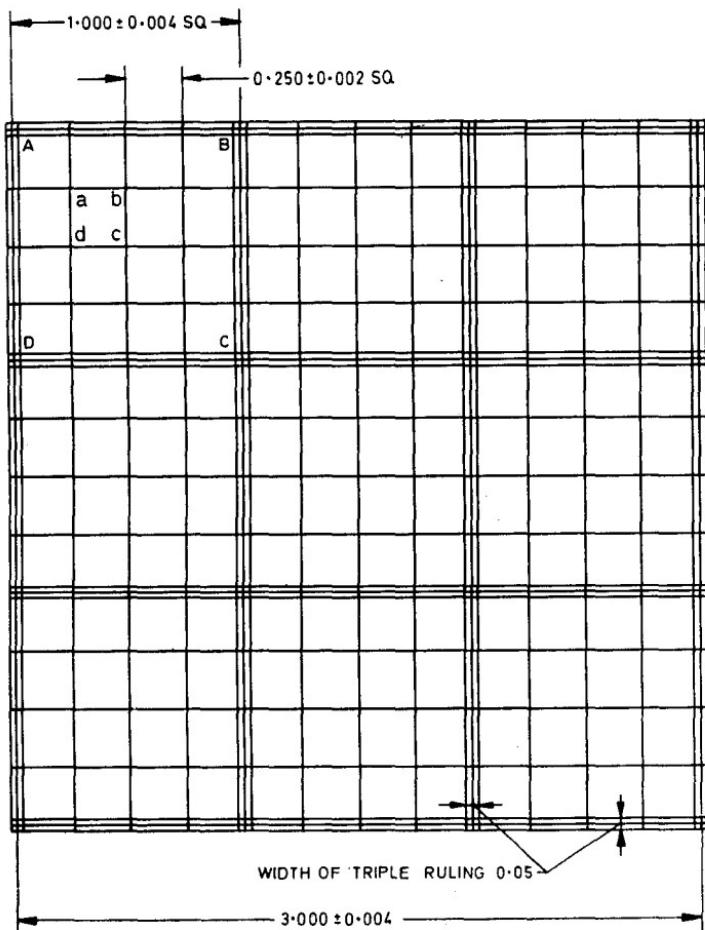
**9.4** The mean thickness of a cover-glass for slides with one or two rulings shall be  $0.45 \pm 0.05$  mm, and for slides with four rulings, it shall be  $0.55 \pm 0.05$  mm. The total variation in thickness for any cover-glass shall not exceed 0.025 mm.

**9.5** When laid on an optical flat and moved about under gentle pressure for a few seconds the cover glasses shall give not more than 5 interference fringes for the smallest size and 7 fringes for the two larger sizes.

**9.6** If dilution pipettes are supplied along with Haemocytometer then pipettes shall conform to IS : 3742-1966\*.

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\*Specification for pipettes, dilution for haemocytometers.



All dimensions in millimetres.

FIG. 9 MODIFIED FUCHS-ROSENTHAL RULING

## 10. MARKING

**10.1** Each slide shall be permanently and legibly marked on the upper surface in accordance with 10.2 to 10.7.

10.2 Either 'Depth 0.1 mm' for slides with Neubauer, Improved Neubauer or Bücker rulings or for slides with Modified Fuchs-Rosenthal with four ruled chambers.

Or 'Depth 0.2 mm' for slides with modified Fuchs-Rosenthal rulings with one ruled or two ruled chambers.

**10.3 Dimensions of small squares ( abcd ) ruled shall be:**

Either '1/400 mm<sup>2</sup>' for slides wth Neubauer or Improved Neubauer rulings.

Or '1/25 mm<sup>2</sup>' for slides with Bürker rulings.

Or '1/16 mm<sup>2</sup>' for slides with Modified Fuchs-Rosenthal rulings.

**10.4 Name of the type of ruling.****10.5 Identification numbers, if required.****10.6 Manufacturer's name or recognized trade-mark.****10.7 IS1 Certification Marking** — The counting chambers may also be marked with ISI Certification Mark.

**NOTE** — The use of the ISI Certification Mark is governed by 'the provisions of the Indian Standards Institution ( Certification Marks) Act and the Rules and Regulations made thereunder. The IS1 Mark on products covered by an Indian Standard conveys the assurance that they have been produced to comply with the requirements of that standard under a well-defined system of inspection, testing and quality control which is devised and supervised by IS1 and operated by the producer. ISI marked products are also continuously checked by IS1 for conformity to that standard as a further safeguard. Details of conditions under which a licence for the use of the IS1 Certification Mark may be granted to manufacturers or processors, may be obtained from the Indian Standards Institution.

**11. PACKING****11.1** Packing shall be done as agreed to between the manufacturer and the purchaser.

# INTERNATIONAL SYSTEM OF UNITS ( SI UNITS )

## Base Units

<i>Quantity</i>	<i>Unit</i>	<i>Symbol</i>
Length	metre	m
Mass	kilogram	kg
Time	second	s
Electric current	ampere	A
Thermodynamic temperature	kelvin	K
Luminous intensity	candela	cd
Amount of substance	mole,	mol

## Supplementary Units

<i>Quantity</i>	<i>Unit</i>	<i>Symbol</i>
Plane angle	radian	rad
Solid angle	steradian	sr

## Derived Units

<i>Quantity</i>	<i>Unit</i>	<i>Symbol</i>	<i>Definition</i>
Force	newton	N	1 N=1 kg. m/s <sup>2</sup>
Energy	joule	J	1 J=1 N.m
Power	watt	W	1 W=1 J/s
Flux	weber	Wb	1 Wb=1 V.s
Flux density	tesla	T	1 T=1 Wb/m <sup>2</sup>
Frequency	hertz	Hz	1 Hz=1 c/s (s <sup>-1</sup> )
Electric conductance	sieniens	S	1 S=1 A/V
Electromotive force	volt	V	1 V=1 W/A
Pressure, stress	pascal	Pa	1 Pa=1 N/m <sup>2</sup>